

AFAMRL-TR-80-40



PRELIMINARY STUDY OF THE EFFECTS OF PROLONGED ACCELERATION ON SPINAL DYNAMICS OF BABOONS

1. Acceleration

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2. Biomechanicai Analysis

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JUNE 1981

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AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY AEROSPACE MEDICAL DIVISION AIR FORCE SYSTEMS COMMAND WRIGHT-PATTFRSON AIR FORCE BASE, OHIO 45488

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animala, "Institute of Laboratory Animala Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTS). At NTS, it will be available to the general public, including foreign nations.

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Director

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+Gz Acceleration Spinal Dynamics	Axial Compressive Loading	Ultimate Load
+Gz Acceleration		Yield Load

spinal dynamics, such as to cause bone strength variations or predisposition to spinal injuries, two baboons were exposed simultaneously to 10 plateaus of ± 4 Gz/30 seconds each separated by intervals of ± 1.5 Gz/45 seconds at the rate

of two times per week for 26 weeks. This phase of the study covers the methodology and compares the results to control non-centrifuged baboons. Subsequently, the spinal columns or these baboons were subjected to extensive

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Block 20. Abstract (cont'd)

mechanical strength testing, the results are given in part 2 of this report.

Part 2: Each vertebra of the two baboons exposed to +Gz acceleration twice/week for 26 weeks (described in part 1) was subjected to axial compressive loading at the rate of 8.89 X 10 to the minus third power meter/sec (21 in./min) on a material testing machine. The data, analyzed by PDP-11/34 computer, were compared with data obtained previously from four non-centrifuged baboons of the same age, weight and sex. Although none of the eight material property (strength tests) evaluated was conclusive, there was a consistent trend that this centrifugation stress has a weakening effect on baboon vertebrae. This preliminary study makes it feasible to repeat the experiment using more animals under better controlled conditions to determine if the spinal changes are significant.

SUMMARIES

PART 1-SUMMARY

During the first phase of this study, the effect of repetitive exposures to acceleration on spinal dynamics of subhuman primates, such as possible bone strength variations or predisposition to spinal injuries, was investigated. In this phase of the program, two young adult male baboons (*Papio anubis*) were lightly anesthetized (tranquilized) with ketamine hydrochloride and placed in specially designed restraint chairs side-by-side on the animal platform of the AFAMRL Dynamic Environment Stimulator (DES), a man-rated centrifuge. They were exposed to 10 cycles of 4G, for 30 seconds with 45-second intervals at 1.5G, twice per week for 26 weeks. Electrocardiograms, heart rate recordings and TV cameras were used to monitor the animals. Following 6 months of acceleration on the centrifuge, the animals were euthanized; and their spinal columns were excised for biomechanical strength evaluation in the second phase of the program.

PART 2-SUMMARY

A review of the literature on the effects of hypergravity on the skeletal system, mainly in lower animals, revealed great differences among different investigators. The study of spinal dynamics of higher animals under centrifugation has been relatively neglected. No previous systematic study was conducted to delineate the influence of acceleration on vertebral bone strength. Consequently, the second phase of the study was conducted to analyze vertebral bone strength of the two baboons that were centrifuged during phase 1. Each vertebra was subjected to axial compressive loading at the rate of 8.89 × 10⁻³ meter/sec (21 inches/min) on a material testing machine. The data were analyzed on a PDP-11/34 computer and compared to data obtained previously from four non-centrifuged baboons of the same age, weight and sex. Eight strength (material property) variables were evaluated: stiffness, ultimate load, displacement to ultimate load, ultimate engineering stress, energy to ultimate load, yield load, displacement to yield load, and engineering yield stress. Although none of the results of these mechanical strength tests was conclusive, there was a consistent trend, indicating that centrifugation at this level and for this time period has a weakening effect on spinal vertebrae of baboons. This preliminary study makes it feasible to repeat the experiment using more animals under better controlled conditions to determine if the vertebral changes are reproducible and statistically significant.

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PREFACE

The research covered in this report was performed at The Air Force Aerospace Medical Research Laboratory (AFAMRL), Wright-Patterson Air Force Base, Ohio, in support of Project 2312V318, "Effects of Prolonged Acceleration of Spinal Dynamics," with Dr. A.R. Slonim as principal investigator.

Part 1 of this study concerned experiments conducted on the Dynamic Escape Simulator (DES). Dr. Sionim is a member of the Biodynamic Effects Branch and Dr. Veghte, Mr. Souder, and Mr. Frazier are members of the Acceleration Effects Branch; both branches are part of the Biodynamics and Bioengineering Division, AFAMRL. The authors acknowledge the valuable assistance of the following in handling the baboons for this study: SSgt Kevin T. Jackson, SSgt Stephen Vinal, A1C Carol Carlson, SSGt David Cushing and others of the Veterinary Sciences Division, Mary Jo Nieser, student aides Charles Silas, George Yewey and Dan Powers, Dr. A.T. Kissen and Mr. Alva A. Karl. Special thanks are due Mrs. Nieser, and Mr. Silas for their long record of assistance throughout this study. In addition, the assistance of Mr. Vance D. Skowronski, numerous military and contractor personnel engaged in operating the centrifuge is gratefully acknowledged. This part of the study was supported by contract F33615-77-C-0515 with Systems Research Laboratories, Inc., and contract F33615-80-C-0500 with Raytheon Corporation.

Part 2 of this study involved the biomechanical testing of each baboon vertebra on a material test system and data analysis on a PDP-11/34. Part 2 was supported in part by contract F93615-78-C-0506 with the University of Dayton Research Institute, Dayton, Ohio. Both authors are members of the Biodynamic Effects Branch of AFAMRL.

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PART 1: ACCELERATION

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INTRODUCTION

The effects of accelerative stress on man have been studied extensively for many years; major emphasis has been on cardiovas-cular, respiratory, metabolic and visual effects. On the other hand, in studies of hypogravity (prolonged bedrest, immobilization and weightlessness), the skeletal system has received major attention. Although the effects of acceleration or hypergravity on bone in animals have been reported in recent years, their implication to humans has not been adequately considered or even mentioned in various literature reviews (cf., e.g., Fraser, 1966; Vasil'yev and Kotovskaya, 1973; Kotovskaya et al., 1977; US Air Force, 1979). The question is raised whether or not personnel who are continually exposed to accelerative forces become more vulnerable to spinal trauma. The new generation of faster, higher-G-performance aircraft have made it increasingly important to determine if repetitive exposures to acceleration have an effect on spinal dynamics (e.g., hone strength variations) or cause debilitating to serious spinal injuries to aircrew members.

An investigation was initiated to study this problem in baboons (Papio anubis), whose spinal geometry resembles that of man. An acceleration profile was selected for the baboons that would be stressful, but at a level low enough to preclude the development of serious physiological disturbances so that testing could be continued throughout a 6-month period. This study consists of two major parts: the acceleration experiment and the postmortem biodynamic evaluation of the spine. Part 1 covers the results of testing two adult baboons simultaneously on a centrifuge twice weekly for six months and comparing them to two control baboons. The results of extensive mechanical stress testing of the baboon vertebrae are discussed in part 2.

TEST ANIMALS

Four adult male baboons of approximately the same size, 22-32 lb (10-15 kg), were selected for this study. The animals were quarantined and maintained in good health at the animal facility of the Veterinary Sciences Division, AFAMRL. Two of them served as test animals by being centrifuged for 6 months, and two served as controls by remaining under observation in the animal facility without exposure to any biodynamic stress during the test period. Early in the study, one control (No. F-18) and one test (No. F-02) baboon were replaced by other baboons. Baboon F-02 severely injured his arm in his cage, requiring surgery. He was replaced by Baboon F-24 in the third week of the program. Control Baboon F-18, because of his very small size, was needed for another experiment and was replaced by Baboon F-20. Nevertheless, the data reported herein cover 26 weeks of observation for all animals under control or test conditions.

The two test baboons were lightly sedated with ketamine hydrochloride in a dose that varied from 100 to 40 mg intramuscularly per animal per experiment throughout the 26-week study. The dose was usually (but not always) at the lower range level as the animal became more adjusted to the study with time. The dose was given just before transporting the animals from the animal facility to the centrifuge building. Here the two animals were brought first into a surgical preparation room and prepared for placement into individual restraint systems, as shown in Fig. 1 (Oloff and Finch, 1978). Following this the baboons were carried immediately to the centrifuge room.

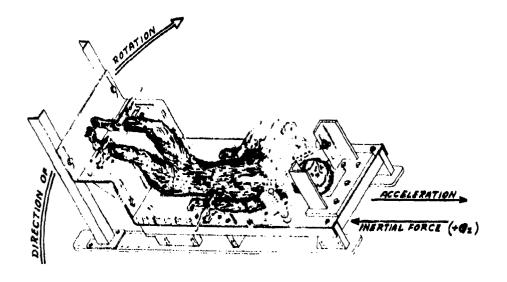


Figure 1. Position of Primate in Restraint System and Direction of Acceleration Load (+ G,)
[modified after AMRL-TR-78-88]

CENTRIFUGE PREPARATIONS

In the preparation room, the two restraint systems were put side-by-side on a large table. Each animal was placed in its assigned restraint system as soon as it was brought into the room. The chests of the animals were shaved and cleaned with alcohol. Medi-Trace® disposable electrocardiographic electrodes were used and arranged in a standard precordial 3-lead placement, with the ground lead over the sternum (MXV₊ positions). Presssigned leads/ cables connected the electrodes to monitoring equipment used in conjunction with the centrifuge, Plastic shields were taped all around the bottom (distal end) of the restraint system to contain urine and feces. Syringes of ketamine (100 mg/ml) were ready if it became necessary to tranquilize the animals further before the test began.

Both harnessed baboons were placed side-by-side (with their restraint systems bolted down) on the animal platform of the centrifuge, the Dynamic Environment Simulator (DES). This platform is located at a radius of 20.5 feet on the DES and opposite the arm that extends to the cab for human subject tests. Because of slight size differences between the two restraint systems, one being a slight modification of the other, and to minimize monitoring errors, each system was placed on the same side of the platform with the same animal throughout the study. A small partition (panel) was inserted between the two systems to reduce distraction between the animals. The assigned (numbered) ECG leads were connected via long cables to the proper amplifier receptacle and checked in the medical monitoring room. The two test baboons in position on the centrifuge, with their ECG electrodes in place, are shown in Fig. 2.



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Figure 2. Baboons in Position on Animal Platform of Centrifuge (DES).

A. One baboon in place on one-half of platform showing his ECG electrodes with wiring.

В

B. Both baboons being centrifuged as seen through camera mounted on arm of centrifuge.

MONITORING

The animals were monitored in the medical monitoring room, overlooking the centrifuge. Electrocardiograms (ECG), heart rate and visual observations were used to assess the physiological state of the animals. The ECG signals went first to a preamplifier located on the animal platform, then through one set of (two) slip rings along the arm of the DES to the monitoring room, where they fed into a processing amplifier. The processing amplifier, which controls the amplitude of the signals from the medical monitor console, processed the output signals to several recording and display devices. One output signal went to a cardiotachometer that counted the heart rate (off the ECG) and converted it to a digital readout on a printer to provide an average 60-second heart rate. Another output was divided and displayed on two oscilloscopes. A third output ted into a Brush (Mark 200, by Clevite) Behannel, strip-chart recorder; this provided a permanent record of the ECG, time and acceleration profile. The option of picking up the output on a tape recorder was not used for this study. A TV camera mounted on the animal platform transmitted pictures through a cable via another set of slip rings to the monitoring room, where they were displayed on several TV screens, A console that presented a digital display on a screen of acceleration in RPM units from start to finish was also used. Figure 3 shows the monitoring equipment.



Figure 3, Medical Monitoring Room Overlooking Centrifuge

- 1. Brush strip-chart recorder
- 2. Cardiotachometers: A. instantaneous H.R. output B. average 60-see H.R. output
- 3. Digital printer (heart rate)
- 4. ECG monitors
- 5. TV monitors
- 6. DES test director's console
- 7. Observation window

ACCELERATION PROFILE

The operation of the centrifuge, or DES, in accordance with a specific acceleration profile was accomplished by a computer program, using a PDP-11, in another room overlooking the centrifuge.

Two baboons were exposed to +G, acceleration simultaneously twice per week for a period of 6 months (26 weeks). This occurred usually at 1:00 PM on Tuesdays and Thursdays of each week. The acceleration profile, shown in Fig. 4, consisted of ten repetitive plateaus of 4 G, (23.5 RPM) each lasting 30 seconds and separated by resting intervals of 1.5 G, for 45 seconds. The onset of acceleration and deceleration was at the rate of 0.3 G per second. At the termination of each centrifuge test, the animals were again sedated to remove their ECG leads, detach their restraint system, and return them to the animal holding facility, the Vivarium, until the next experiment. After the second centrifuge run of the week, however, radiographs of the skeletal system were taken of each animal; this was accomplished weekly for the duration of the study.

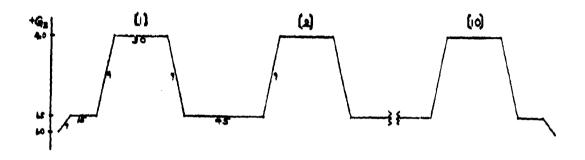


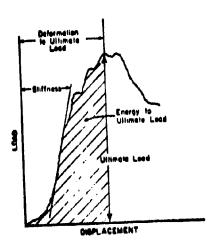
Figure 4. Acceleration Profile. (Numbers under curve represent time in seconds; those in brackets represent peaks 1-10 at + 4 G_o)

RESULTS AND DISCUSSION

The two test baboons tolerated well the exposure to the 4 G, acceleration profile twice weekly for 26 weeks. As the animals became more adjusted to the experiment, the amount of ketamine necessary for sedation at the start of a centrifuge run was reduced about 50%, from 100 to 50 mg i.m., after 6 weeks; occasionally, the dosage had to be increased to the 60-80 mg level, or one baboon (F-32) required 10-20 mg more than the other (F-24) in four of the centrifuge tests in the last month. Both animals exhibited erratic electrocardiograms during acceleration, which was especially pronounced over the first few months of the study. The cardiovascular data on these same baboons are currently being evaluated; any significant findings will be reported later.

Routine anterior/posterior and lateral X-rays were taken of the test baboons, usually after the second centrifuge run of the week. The X-rays verified that both test animals were approximately the same age; each showed two molars (on the right side) that had not surfaced through the gum. The control baboons also fit in this category. One of the test baboons (F-24) exhibited an enlarged heart that became more pronounced with time. The same baboon also showed radiologically some spinal changes, such as a loss of intervertebral disc space height, an approximation of the posterior vertebral surfaces and, generally, a loss of border alignment from the T₄ to T₁₈ spinal levels. This radiological observation was not clearly demonstrated at all times. Some disparity in the X-rays was noted at the transition line of the spinal column due in part to hyperextending the baboon spinal column on the X-ray table and due sometimes to the questionable quality of some of the radiograms. Therefore, a standard radiographic procedure that incorporates stricter control of body positioning for X-ray evaluation will have to be instituted in a follow-on study.

In view of the implication of the preceding radiological observation to human subjects undergoing similar long-term exposures to acceleration, a thorough biodynamic evaluation of the spines of these baboons appeared warranted and was undertaken (as presented in part 2). The spinal columns of the test baboons (F-24 and F-32) were excised and cleaned of ligamentous attachments, etc.; and the vertebrae T_1 to L_6 were each strength tested at one loading rate (8.89 \times 10⁻³ meter/sec) and grouped tachments, etc.; and the vertebrae T_1 to L_6 were each strength tested at one loading rate (8.89 \times 10⁻³ meter/sec) and grouped also into six column positions for data analysis. The results were compared to the data obtained previously from four non-centrifuged baboons of the same age, sex and weight. The following dependent variables concerning the material properties of the thoracic and lumbar vertebrae, $T_1 - L_6$, were analyzed and compared as a function of column position: stiffness, ultimate load, displacement to ultimate load, ultimate engineering stress, energy to ultimate load, yield load, displacement to yield load, and engineering yield stress. A typical test curve is shown in Fig. 5; the test specimen load is plotted, as ordinate, versus the specimen displacement, as abiscissa (Kazarian and Graves, 1979). The aforementioned material property characteristics of the vertebrae of the two test baboons were analyzed by a mechanical testing machine and compared to those of the non-centrifuged baboons. The detailed results are reported in part 2 of this study. We think it is relevant, therefore, to repeat/extend this experiment using more baboons under better controlled conditions to increase the sample size and confirm that any spinal changes are real, not drug-induced, and statistically valid.



[•] For more detail see Figure 6.

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PART 2: BIOMECHANICAL ANALYSIS

A. R. Slonim, PhD L. E. Kazarian, Dr Ing

INTRODUCTION

The effects of a hypergravity environment on the skeletal system have received major attention only within the past 15 years or so. Many of these studies generally have been in agreement with Wolff's Law of Bone Growth, viz., that an increase in stress to bone results in growth of its stressed elements. However, the results reported in the literature by various investigators using mainly rodents and birds exposed to centrifugation have been quite varied, contradictory in some cases, and very complex especially in regard to bone alterations. The reasons for this may depend on the nature and extent of the hypergravity state; the animal species, age, sex and bone remodeling rate; and, to some extent, the control conditions of the study. The complex differential response of bone to gravity and some of the control closer reported in the literature are discussed.

S. D. Smith (1975) using 3-month-old rats, raised as a third generation of constantly centrifuged animals, and two different groups of control rats, one for earth (I G) and one for rotation (1.03 G), showed the following differential response to femure of rats exposed to 2 G: a decreased length and length/diameter ratio, increased cortical thickness/diameter ratio, and ossification of the femur head being slightly advanced but the distal epiphyseal plate being thinned. Negulesco and Clark (1976) found that 1-week-old female chicks (Rhode Island Reds) exposed to 2 G showed a decreased fractured radial length, weight and a smaller proximal epiphyseal diameter. Negulesco (1976) reported also that there was a significant decrease in the average weight of both intact and fractured radii of female chicks exposed to 2 G for 2 weeks. Note that the length of intact radii was no decreased as were fractured radial length, weight and epiphyseal-diaphyseal diameter in the Negulesco study. This conflicts with the results of Oyama and Zeitman (1967) and S. D. Smith (1975), who reported that intact femoral length of rat was decreased by chronic centrifugation, and A. H. Smith (1972), who reported that humeral length of chickens increased rather than decreased upon chronic acceleration. Negulesco (1976) attributed the difference in results to the use of older animals and longer centrifugation time. A. H. Smith and Kelly (1963), who also worked with chronically centrifuged chickens, reported that femoral size increased but osseous mass was smaller than that of control animals. Except for differences in length of intact radii, Negulesco's work generally supported the findings of Oyama and co-workers (1967, 1973), who reported that a decreased femoral mass, length, midshaft diameter and body weight occurred in chronically centrifuged female rats. On the other hand, Jankovich (1971) stated that bone development of rats as a function of age appeared unaffected by 'ow G forces from 1.5 to 2.5 G; however, he did observe a slower longitudinal bone growth in these centrifuged rats. Negative (no effect) results were reported also for chickens by Riggins and Chacko (1977), who exposed Single Comb White Leghorn adult males to centrifugation varying up to 3 G over an 18-week

Wunder and co-workers in a series of studies evaluating the femur-bending properties of hypergravity reported that young male rats (5-8 wks old) centrifuged to 3 G for up to 65 days increased their ability to sustain bending forces, which was attributable to increased strength of material rather than bone size or shape and to young age where experimental bone material seemed to grow to maturity better than in mature rats (Wunder, Cook et al., 1977). This same 3-G stress also increased the femur-bending properties in mice, but had less effect (50%) on the smaller animals (mice) than on rats; i.e., the femurs were not as large or as strong in mice as in rats (Wunder and Welch, 1977). Furthermore, mice at 4 G exposure did not show the same effect upon the femur's supporting ability as mice at 3 G. The 4 G mice (as with 3 G rats and mice) showed relatively weaker male bones but larger size than the controls; thus, the 3 G femurs should be better able to support decreased body mass in mice than the 4 G femurs. It appears that the optimum field for development of the femur's supporting ability is below 4 G with mice and may be below 3 G; this has led Wunder to propose further studies to establish a linear range of this "effective vs. field-intensity relationship." Wunder (1977) also reported that a greater degree of femoral weakness under excessive hypergravity existed with male than with female mice.

S. D. Smith (1977), using earth and rotation controls and rats of both sexes as previously (1975) except not derived ("selected") from three generations of centrifuged rats, reported that 2 G up to 16 weeks caused decreased femoral length, reduced femoral diameter, decreased L/D ratio, increased femur length/body weight, decreased cortical thickness, increased diameter/cortical thickness ratio, thinned and distorted epiphyseal plate, and thickened condylar cartilage in female rats. Some of these changes, such as in diameter and cortical thickness, were pronounced in the early stages of the study for both sexes (i.e., up to 4 wks), after which the effects were greatly reduced in females. The rotation controls (1.05 G) exhibited opposite changes to the centrifuged rats in that they exhibited increased femoral length, increased L/D ratio, decreased diameter/cortical thickness ratio, and accelerated ossification of femoral head; similarly, the 1.05 G rats showed a reduced femoral diameter as did the 2 G rats. In addition to the sexually dimorphic response of these rats, with the females being more severely affected than males in many (not all) skeletal areas by hypergravity, Smith stated that rotation and hypergravity produce opposite effects on growing animals, with the former enhancing growth and the latter retarding it, rotation seems to advance the formation of ossification centers while hypergravity seems to depress the function of the epiphyseal plates. He emphasized that hypergravity and rotation appear to have both a qualitative and quantitative difference in young versus adult animals.

Negulesco and Kossler (1978), continuing the studies with newly hatched Rhode Island Red chicks, reported that 2-week-old chicks exposed to 2 G for 2 weeks developed an increased width of cartilage layers of the proximal epiphyses and inhibition of both height and width of the cartilage layers of the distal epiphyses. In a more recent report on Rhode Island Red fowl, Smith, Spangler, Burton and Rhode (1979) studied the response of mature males to 6 G/4 min, eight times/day, five times/week for 24 weeks. Based on mortality rates, lymphocyte frequency (a stress indicator) and postmortem findings, they postulated that animals can be divided into three categories: a very susceptible group, a moderately tolerant group and a rather resistant group, with the first group showing the most severe lymphopenia, morbidity, pathology, mortality and large body mass. This indicates that the same animal species are heterogenous in their response to repeated accelerations.

A histomorphometric study on bone remodeling in 3-week-old female rats exposed to 2 C for a total of 18 days was reported recently by Nogues and Peuchmaur (1980). Following centrifugation, all bones were measured, growing cartilage was studied on decalcified sections, and histomorphometric and histodynamic (tetracycline fixation) studies were conducted on calcified sections hy analyzing six bone parameters (e.g., bone volume, relative osteoid surface, mean osteocyte lacunae surfaces, resorption lacunae, etc.) The major changes noted were a shortening of femurs associated with growth cartilage alterations, a decrease in bone volume without an increase in osteocytic activity, and (by tetracycline fluroescent analysis) a reduced appositional rate in bone. The bone remodeling/equilibrium results, in which there were deficient bone formation (apparently due to slower osteoblastic activity) and little if any bone hyperresorption, were in disagreement with the "classical blomechanics data according to which reduced bone mass is a response to reduced mechanical force and inversely." The histodynamic, equilibrium and other changes observed in this study that were in opposite directions reflect that numerous factors are involved in hypergravity effects on bone remodeling. In this regard, e.g., S. D. Smith (1975, 1977) pointed out that rotation may be important also at least qualitatively and tended to act in an opposite direction to acceleration. Nogues and Peuchmaur believe further that the osteoporosis seen in their centrifuged rats is due primarily to stress acting on ACTH, which, in turn, releases corticosteriod hormones, for the response is similar to that produced by hyperactive adrenals or cortisone (e.g., opposition to conjugation cartilage development, reduced bone formation followed by reduced resorption in transcular bone first, and reduced intestinal absorption of calcium plus increased urinary calcium excretion). These workers found little or no role played by parathormone. In contrast, Sannes and Hayes (1975) showed increased parathyroid gland secretory activity in Mongolian gerbils exposed to continuous acceleration to 2 C for 60 days. Recently, other hormones have been implicated in hypergravity stress. Florindo and Negulesco (1980) reported that acceleration on 2-week-old chicks did not affect the growth hormone content of the anterior pituitary, but markedly reduced the prolactin levels of this gland. Even in the area of calcium levels of centrifuged animals, discrepancy exists. Whereas Oyama and Zeitman (1967) reported that rats centrifuged at 4.7 G for one year showed depressed calcium levels, Sannes and Hayes reported no significant calcium change in gerbils centrifuged at 2 G for 60 days; perhaps in the former case the hypergravity was excessive. It is quite clear from all the above reports that great differences in results, even contradictions, exist among the different investigations. To a large extent these differences are due to variations in experimental conditions, such as animal species, age and sex, magnitude and duration of acceleration, the dynamic equilibrium state of bone, and the time frame in which measurements are taken.

Although considerable work still remains to clarify the hypergravity effects observed above in lower animals, there is nothing in the literature concerning the effects of prolonged acceleration on the skeletal system of higher animals, especially at the subhuman primate level. This is unfortunate, for humans are now accumulating long exposure time to accelerative stress since the advent of high performance aircraft as discussed in part 1 of this report. Recently, Carlson and Zackrisson (1977) reported that Swedish flying personnel (average of about 4000 hours), who were examined at 5- and 10-year intervals, exhibited a loss of alveolar marginal bone of the mandible. High altitude flying, reduced partial pressure of oxygen, stress and vibration were suspected as possible causes. It seems propitious now to examine the possible skeletal effects of prolonged acceleration on humans. No previous systematic investigations have been conducted that delineate the influence of centrifugation on vertebral bone strength. Part 2 covers the results of biomechanical testing, i.e., axial compressive loading, of the vertebral bodies of the spines of the two baboons exposed simultaneously to G, acceleration for 6 months. The results were compared to previous strength values collected on baboons of similar age, weight and sex.

METHODS

ACCELERATION

Two young, adult male baboons (10-15 kg) were centrifuged simultaneously to 10 plateaus of 4 G, for 30 seconds separated by intervals of 1.5 G, for 45 seconds at the rate of two times per week for 26 weeks. The animal care, preparation, and centrifuge experiments were described in part 1.

TEST SPECIMEN

The vertebral columns of the centrifuged baboons (after euthanasia) were excised en masse, identified and stored in a freezer at -30° centigrade. Thirty-six hours before testing, the vertebral columns were removed from the deep freeze and allowed to partially thaw. Simultaneously, individual vertebrae were disarticulated from one another by slicing through the midsection of the intervertebral disks, the articular capsules were sectioned, and the vertebral bodies were cut away at the base of the pedicles using a band saw. Each individual vertebral centrum was cleaned of all tissue adhering to its surfaces. Care was exercised so the surface of the cortical bone was not marred. The remains of the intervertebral disk (annulus fibrosus, nucleus pulposus and cartilaginous end-plate) were carefully removed from the superior and inferior vertebral body surfaces.

The superior and inferior vertebral bearing surfaces were photographed. The vertebral body bearing areas were determined for both the superior and inferior surfaces; using the photographs, the surface area measurements were averaged. The height of the vertebral centrum was measured. To promote a uniform load distribution, the bearing surfaces of each vertebral centrum were potted in an acrylic compound. Using dental acrylic resin, the potting produced circular-shaped pots with the specimen located centrally. The diameter of the pot was subsequently used to locate the center of the specimen coincident with the loading axis of the test machine. The vertebral centra were placed into the acrylic at both ends, and the entire assembly was placed in a V-shaped trough to assure that both surfaces were kept parallel and axially lined as previously described (Kazarian and Graves, 1979). The vertebral centra were wrapped in a towel in Ringer's solution to prevent drying while the acrylic curad.

TEST MACHINE

An electrohydraulic closed loop test machine (Model 810 Material Test System, MTS System Corp., Minneapolis, Minn.) was used to strain each test specimen (vertebral centrum). The system is centered around an electrohydraulic closed loop test machine capable of being programed and controlled in load, strain and displacement. With the machine in the displacement control mode, a linear ramp function was used to strain each test specimen. The imposed time-dependent displacement and the resultant compression loads were recorded. Ram displacements were measured using a linear variable differential transformer, while the specimen reacted against a four-arm bridge strain gauge load cell. A multichannel FM magnetic tape recorder and a multichannel transient recorder were used to store the test results. Load and displacement data were stored in the digital memory of a transient recorder for playback at reduced speeds into the X-Y recorder. A test fixture chamber was designed for observing and photographing each test specimen (Kazarian and Graves, 1979).

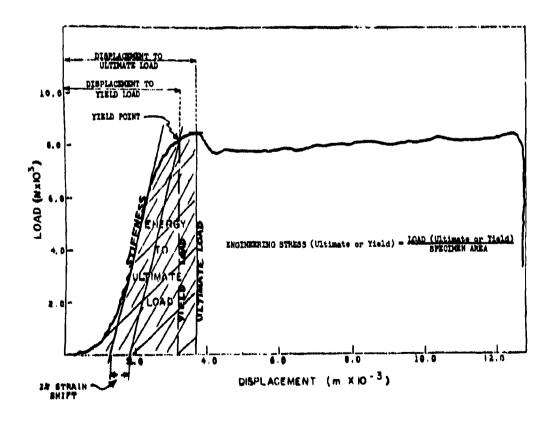


Figure 6. Typical Load vs. Displacement Curve

STRENGTH VARIABLES

A typical load versus displacement curve is shown in Fig. 6. As the specimen is stressed from the start, the relationship between load and displacement is relatively linear, reflecting the elastic nature of the specimen. Beyond this region, as loading continues, the specimen becomes less elastic (less stiff) and deformation changes from the reversible to irreversible state. A line calculated by a least squares fit of the linear portion (slope) of the elastic section of the load vs. displacement curve is defined as the stiffness of the specimen. On the curve beyond the apparent linearly increasing elastic section, corresponding to the maximum load value (where a tangent to the curve becomes parallel to the displacement axis), is the point the distance from which perpendicular to the abscissa is defined as the ultimate load; it is also the point where damage to the specimen becomes irreversible. The amount of displacement from zero to where the ultimate load intersects the abscissa is displacement to ultimate load. The energy to ultimate load is defined as the area under the load vs. displacement curve, from the point of origin (zero displacement) up to the ultimate load line. The ultimate engineering stress is computed by dividing the ultimate load by the cross-sectional area of the apecimen.

The yield load differs from the ultimate load in that at the ultimate load an apparent structural failure has occurred within the specimen; at the yield load, it is assumed that adverse structural changes are occurring within the specimen but the damage is reversible. The yield load starts at a point on the load vs. displacement curve that deviates from the apparent elastic portion (stiffness) of the loading curve. For specimens from fresh, young primates (non-brittle), a 2% displacement deviation (strain) has proved to give satisfactory results. The yield point (on curve) is determined analytically by taking 2% of the specimen pretest height (2% strain) and shifting the stiffness line to the right; the point at which it intersects the curve is the yield point. A perpendicular time from the yield point to the displacement axis is the yield load. The amount of displacement from zero to the yield load (a line perpendicular to the abscissa) is the displacement to yield load. The yield stress is computed by dividing the yield load by the specimen cross-sectional area.

SPECIMEN ANALYSIS

The individual vertebral bodies of the spinal columns of the two centrifuged baboons, F-24 and F-32, were tested from the T1 to L6 levels and at one compressive loading rate of 8.89×10^{-3} meter/sec (21 inches/min). Since it is reasonable to assume that differences between adjacent vertebral bodies are insignificant, the spinal column was apportioned equally to six column positions, each composed of three adjacent vertebrae as follows:

Column Position	Assigned Vertebral Bodies
Pi	T1, T2, T3
P2	T4, T5, T6
P3	T7, T8, T9
P4	T10, T11, T12
P5	L1, Ĺ2, L3
P6	L4, L5, L6

The material properties data of each vertebral body were averaged per column position. A more meaningful comparison could be made between experimental conditions (centrifuge vs. non-centrifuge) when comparing the data as a function of column position rather than individual vertebral levels. Thus, the centrifuged data were compared with the data obtained previously from the four non-centrifuged baboons of the same age, weight and sex.

The non-centrifuged baboon vertebral body specimens were subjected previously to three different loading rates: 0.21, 21 and 2100 inches/min. In order to accomplish this with a single axial compressive load per specimen (centrum), the specimens of all four non-centrifuged baboons were distributed randomly in a multi-species vertebral body test matrix, so that each column position was represented per baboon per loading rate. Thus, each column position had at least one of its three component bodies tested at one of the three rates. For the purpose of this comparative study, only the data obtained at the same loading rate as for the centrifuged baboons (21 inches/min) are examined. The non-centrifuged vertebral centra were randomly distributed in the matrix as follows:

Baboon	Column Position					
No.	Pl	P2	P 3	P4	P5	P 6
F-72	71	T4	-18	T12	L3	1.5
F-76	Т3	T6	Т9	T10	1.2	1.4
F-86	Т3	T4	T8	TII	LI	L5
F-78	T2	T5	T7	T11	1.1	1.6

The data from the non-centrifuged baboons were analyzed per column position per animal; then the data from each animal were combined to give an average value for each column position. This average was used to compare with the column position average of the two centrifuged baboons. It would have been possible to compare some individual vertebal levels between experimental conditions, but not all because each vertebra of the same non-centrifuged baboon was not tested at the same loading rate, a requirement to obtain and compare data for a whole spinal column. Comparing individual vertebrae between such a small number of animals is less attractive also because the variability between such few baboons would be greater than between individual units of the same column or column position.

The following dependent material properties were analyzed first as a function of each vertebral body and then averaged for each column position per centrifuged baboon. The column position data were next combined to give average values for both centrifuged subjects.

Dependent Variables

- Stiffness · N/M
- Ultimate Load · N
- Displacement to Ultimate Load · M
- Ultimate Engineering Stress · PASCALS
- Energy to Ultimate Load JOULES
- · Yield Load · N
- Displacement to Yield Load M
- Engineering Yield Stress · PASCALS

Each dependent variable (average for the centrifuged baboons) was plotted as a function of column position and compared graphically to that obtained for the four non-centrifuged baboons. If it is assumed that combining the data from all subjects will minimize the differences between them, then column position, itself, remains as the only independent variable in the study.

Since there were only two test baboons in this study, the data were not statistically evaluated, but observed for consistent trends within the test subjects and between the two experimental groups and for determining the direction of further efforts in this program.

RESULTS AND DISCUSSION

The results are described separately for each of the dependent variables. They are evaluated first as a function of vertebral body from T1 to L6 then column position from P1 to P6, and last compared between centrifuged and non-centrifuged baboons. The individual curves on the centrifuged baboons (F-24 and F-32) are located in the Appendix.

STIFFNESS

Stiffness gradually increased from the T1 to L6 vertebral levels for Baboons F-24 and F-32, respectively. Fig 7 presents the average curve for both centrifuged baboons. In terms of column position, stiffness increased from P1 to P6 for the individual centrifuged baboons; the average curve for both is shown in Fig. 8. This increase in stiffness is expected with increasing geometry of vertebral centrum from the T1 to the L6 levels of the spinal column. (Stiffness vs. vertebral level and vs. column position per centrifuged baboon are found in the Appendix.)

When a comparison was made between the two centrifuged baboons and the four non-centrifuged baboons, the centrifuged curve was slightly less stiff from P1 to P6, as shown in Fig. 9. This indicates that the centrifuged vertebrae were less resistant (i.e., weaker) to compressive loading than the non-centrifuged vertebrae.

ULTIMATE LOAD

Ultimate load did not increase appreciably between the T1 and T5 levels for either test baboon (see Appendix); from T5 to 1.6, however, the curves for both baboons increased sharply. The average curve of ultimate load vs. vertebral level for both baboons is shown in Fig. 10. The response in terms of column position, likewise, showed no change between the first two column positions, but a sharp increase from P2 to P6 for either test baboon (see Appendix). The average ultimate load vs. column position curve is shown in Fig. 11.

When ultimate load, or load to failure, was compared between the two experimental groups, as shown in Fig. 12, the curve for the centrifuged baboons is below that of the non-centrifuged baboons, as was the case with stiffness. Although in both curves ultimate load increased with column position, the curve for the centrifuged baboons was flatter, indicating that less load was required to fail the centrifuged specimens.

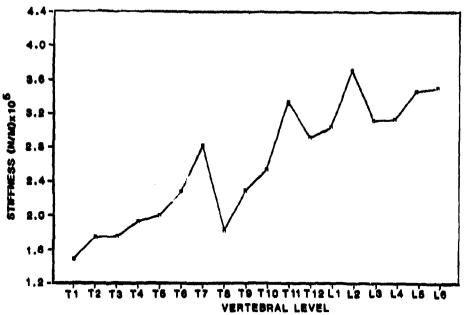


Figure 7. Stiffness vs. Vertebral Level for Centrifuged Baboons F-24 and F-32.

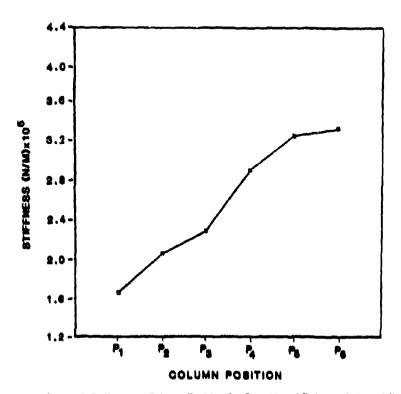


Figure 8, Stiffness vs. Column Position for Centrifuged Baboons F-24 and F-32.

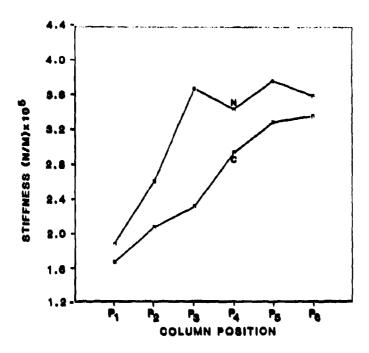


Figure 9. Comparison of Stiffness vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

DISPLACEMENT TO ULTIMATE LOAD

Displacement to ultimate load, or decrease in vertebral centrum height to point of failure, actually decreased at the beginning from the T1 to T7 levels, then gradually increased from T7 to L6 in each of the two test baboons. The average curve of displacement to ultimate load as a function of vertebral level for both centrifuged animals is shown in Fig. 13; the curve shows in part a diphasic response: a decrease to the T7 level followed by an increase to the L6 level. When the response was compared as a function of column position, each test baboon showed a decrease from P1 to P2 position, followed by an increase from P3 to P6. This is evident in Fig. 14, where the average data for both baboons were plotted vs. column position. The diaphasic response reflects in part no change in ultimate load between the first two column positions (Fig. 11).

When displacement to ultimate load was compared between the centrifuged and non-centrifuged baboons, as shown in Fig. 15, the curve for the centrifuged baboons was slightly less (lower and flatter) than for the non-centrifuged baboons, especially from the P2 to P6 column positions, somewhat similar to ultimate load. The trend indicated again that less load was required to permanently damage the centrifuged specimens.

ULTIMATE ENGINEERING STRESS

The response of ultimate engineering stress, or force per unit area, as a function of vertebral level was not very clear or as apparent as the above variables. Each centrifuged baboon showed a response that decreased from T1 to T2, then increased from T2 to T3, followed by a sharp decrease to T4; the curve leveled off and gradually decreased from T4 to L6 in one test animal (F-32), but increased up to T9 then decreased to L6 in the other (F-24). The average response for both baboons (Fig. 16) showed the curve to decrease from T1 to T2, then increase to T3, followed by a sharp decline from T3 to T4, which gradually built up (increased) to a small peak at T9; from T9 to L6 there was a gradual decrease in stress. When the data were evaluated in terms of column position, ultimate engineering stress showed a decrease from P1 to P2 in each of the centrifuged animals. However, in F-24, it increased from P2 to P3 and then dropped sharply from P3 to P6; whereas in F-32, the response showed a leveling off and gradual decline from P3 to P6. When the combined data were plotted vs. column position, the curve (Fig. 17) showed a sharply drop from P1 to P2, followed by a slight increase at P3; the stress was sharply decreased from P3 to P6.

When ultimate engineering stress vs. column position was compared between the two experimental groups, the curve for the centrifuged baboons was lower than for the non-centrifuged baboons, as seen in Fig. 18. The apparent trend indicates that the force per unit area was less for the centrifuged vertebrae. If it is assumed that the average vertebral body areas were relatively the same between column positions in both experimental groups, this response would be reflected in the ultimate load being less in the centrifuged than non-centrifuged animals.

ENERGY TO ULTIMATE LOAD

Energy to ultimate load tended to decrease at the upper thoracic levels from T1 to T4 or T5, but generally increased thereafter to the L6 level for either centrifuged baboon. The average curve for both baboons (Fig. 19) showed this initial decrease, followed by a gradual increase as vertebral level increased to L6. The energy vs. column position curve showed a decrease from P1 to P2 followed by an increase from P2 to P6; the latter increase was gradual for Baboon F.24 and more abrupt for Baboon F.32 with increasing vertebral level. The average curve for both baboons (Fig. 20) showed a decrease between the first two column positions followed by a steady increase from P2 to P6.

When the energy to ultimate load vs. column position was compared between centrifuged and non-centrifuged animals, as shown in Fig. 21, the average curve for the two centrifuged baboons was lower and flatter than the non-centrifuged animals; thus, the effect was not as prominent for the test animals. The trend observed indicates that less energy was required to fail or permanently damage the centrifuged vertebras.

YIELD LOAD

The yield load showed in general a gradual increase from T1 to L6 for either test baboon. The average curve for both baboons (Fig. 22) showed a gradual increase of yield load as a function of vertebral level. In terms of column position, the yield load increased steadily from P1 to P6; the average curve for both baboons showing this gradual increase of yield load vs. column position is shown in Fig. 23.

When yield load vs. column position was compared between the two centrifuged and four non-centrifuged haboons, the curve for the centrifuged animals was lower than that for the non-centrifuged ones, as seen in Fig. 24. This response is parallel to that for ultimate load and reflects here that less load was required to cause reversible damage to the centrifuged specimens.

DISPLACEMENT TO YIELD LOAD

The response of displacement to yield load vs. vertebral level was not very clear or consistent except to show a slight tendency to increase with increasing vertebral level for either test baboon. The average displacement to yield curve for both baboons is shown in Fig. 25. When displacement was evaluated in terms of column position, one baboon (F-24) showed a consistent increase with column position that was more apparent than in the case of the other baboon (F-32). The average displacement to yield curve (Fig. 26) reflects this inconsistency, but generally indicates a trend of increasing displacement to yield with increasing column position.

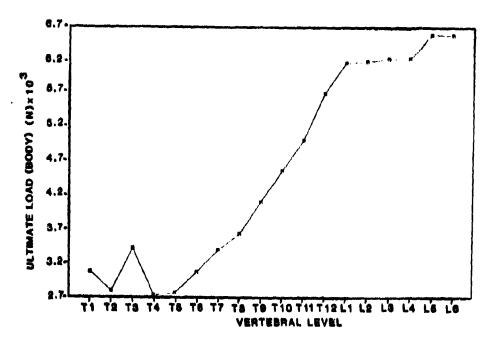


Figure 10. Ultimate Load vs. Vertebral Level for Centrifuged Baboons.

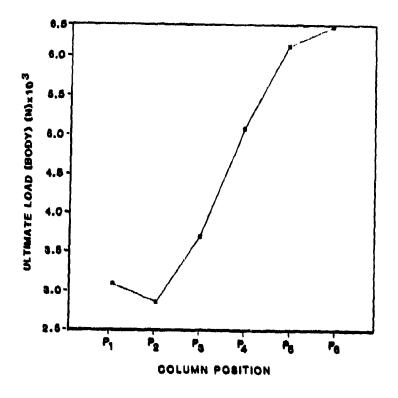


Figure 11. Ultimate Load vs. Column Position for Centrifuged Baboons.

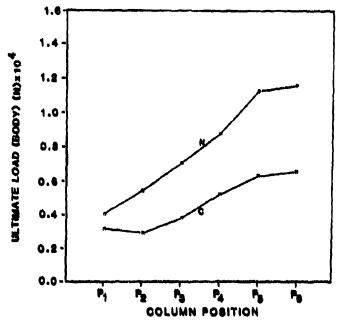


Figure 12. Comparison of Ultimate Load vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

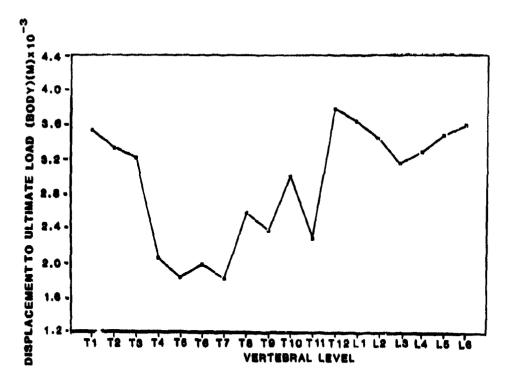


Figure 13. Displacement to Ultimate Load vs. Vertebral Level for Centrifuged Baboons.

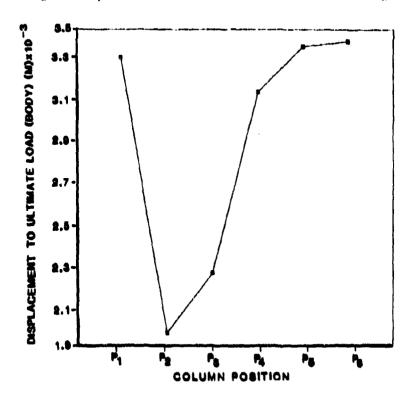


Figure 14. Displacement to Ultimate Load vs. Column Position for Centrifuged Baboons.

When the centrifuged versus non-centrifuged displacement to yield load curves were compared, as shown in Fig. 27, the difference was not too apparent. The centrifuged curve was slightly lower than the non-centrifuged one for most of the column positions (except P4), thus showing a trend similar to that observed for displacement to ultimate load above.

ENGINEERING YIELD STRESS

The response of engineering yield stress vs. vertebral level was creatic (up-down-up) from T1 to T9, then tended to decrease from T9 to L6 for both test baboons. The average yield stress curve reflects this response, as shown in Fig. 28. The yield stress vs. column position curve was different for both centrifuged animals from P1 to P3; however, both animals showed a decrease in yield stress from P3 to P6. This is evident in Fig. 29, which is the average curve of yield stress vs. column position for both test baboons.

When engineering yield stress was compared between the two test baboons and the four non-centrifuged baboons, both curves (Fig. 30) showed little change from P1 to P3 followed by a decrease from P3 to P6. However, the curve for the centrifuged baboons was lower than that for the non-centrifuged animals. As with ultimate engineering stress, the apparent trend indicates that the force per unit area was less for the centrifuged vertebrae.

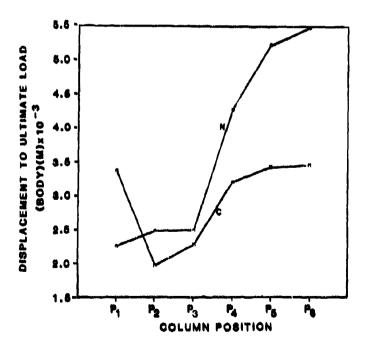


Figure 15. Comparison of Displacement to Ultimate Load vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

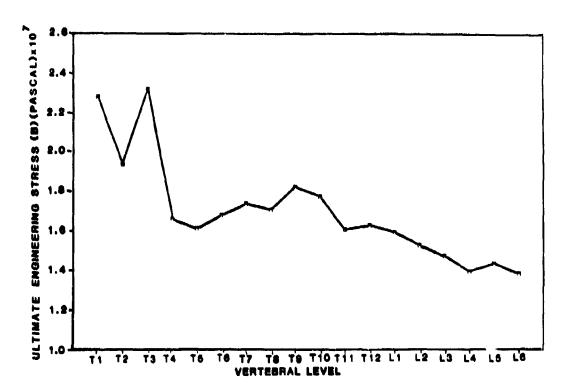


Figure 16. Ultimate Engineering Stress vs. Vertebral Level for Centrifuged Baboons.

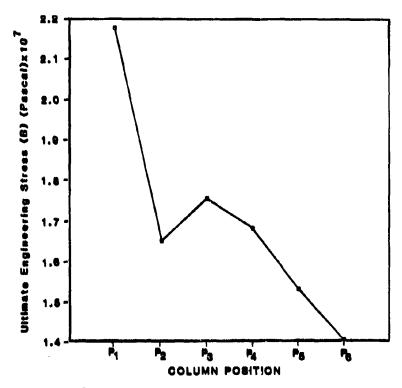


Figure 17. Ultimate Engineering Stress vs. Column Position for Centrifuged Baboons.

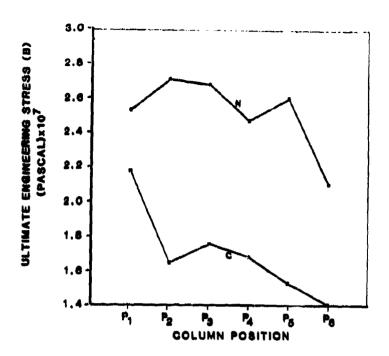


Figure 18. Comparison of Ultimate Engineering Stress vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

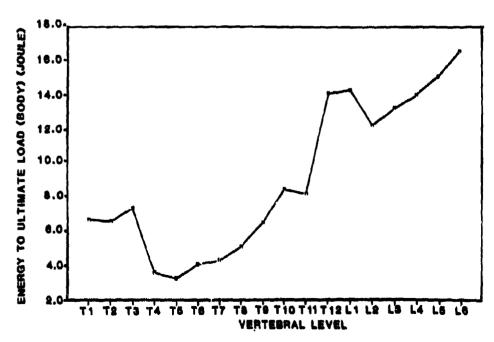


Figure 19. Energy to Ultimate Load vs. Vertebral Level for Centrifuged Baboons.

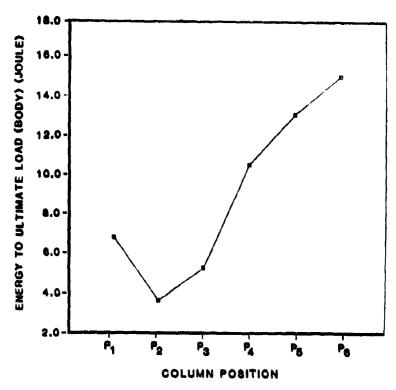


Figure 20. Energy to Ultimate Load vs. Column Position for Centrifuged Baboons.

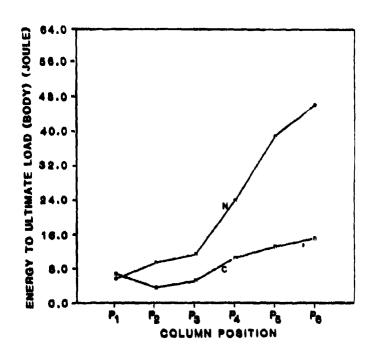


Figure 21. Comparison of Energy to Ultimate Load vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

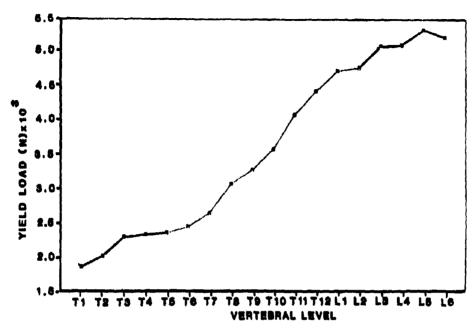


Figure 22. Yield Load vs. Vertebrai Level for Centrifuged Baboons.

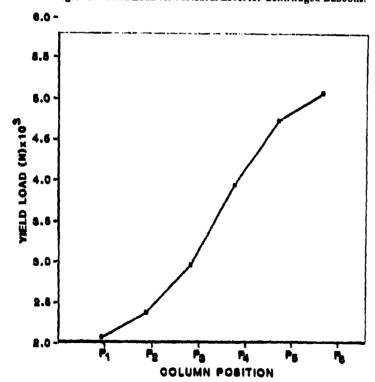


Figure 23. Yield Load vs. Column Position for Centrifuged Baboons.

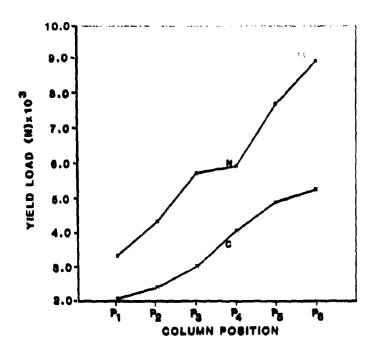


Figure 24. Comparison of Yield Load vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

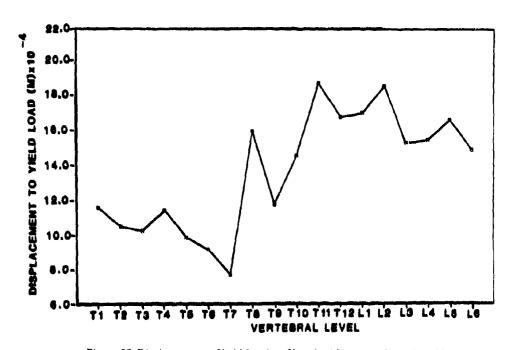


Figure 25. Displacement to Yield Load vs. Vertebral Level for Centrifuged Baboons.

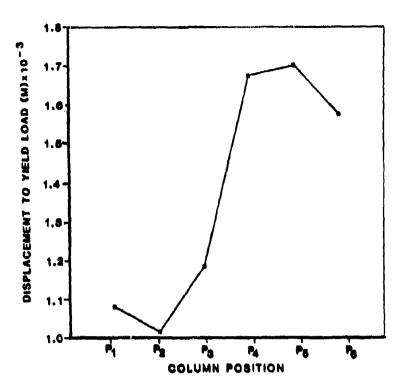


Figure 26. Displacement to Yield Load vs. Column Position for Centrifuged Baboons.

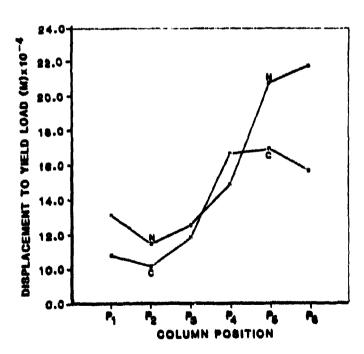


Figure 27. Comparison of Displacement to Yield Load vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

GENERAL COMMENTS

Although the results of the mechanical strength tests were not conclusive for any of the material properties considered, there was a consistent trend indicating a weakening effect on the vertebrae of the baboons exposed to this acceleration stress during a 26-week period. This preliminary study makes it feasible to repeat the experiment using more animals under better controlled conditions so as to determine if the spinal vertebral changes due to prolonged acceleration are reproducible and statistically significant.

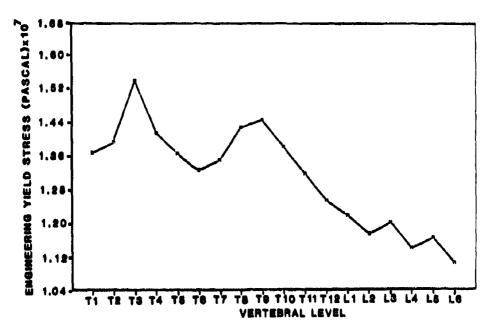


Figure 28. Engineering Yield Stress vs. Vertebral Level for Centrifuged Baboons.

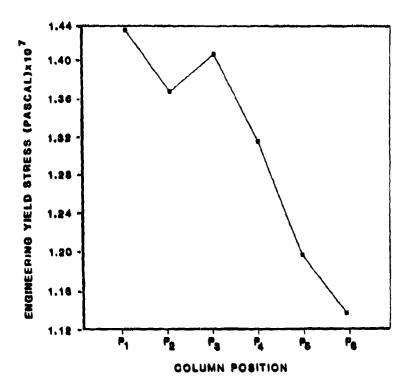


Figure 29. Engineering Yield Stress vs. Column Position for Centrifuged Baboons.

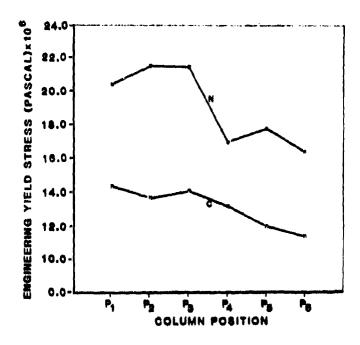
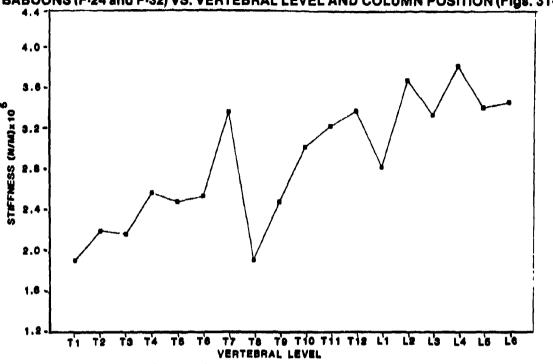


Figure 30. Comparison of Engineering Yield Stress vs. Column Position between Centrifuged (C) and Non-centrifuged (N) Baboons.

APPENDIX

INDIVIDUAL MATERIAL PROPERTY (STRENGTH) CURVES OF CENTRIFUGED BABOONS (F-24 and F-32) VS. VERTEBRAL LEVEL AND COLUMN POSITION (Figs. 31-62)



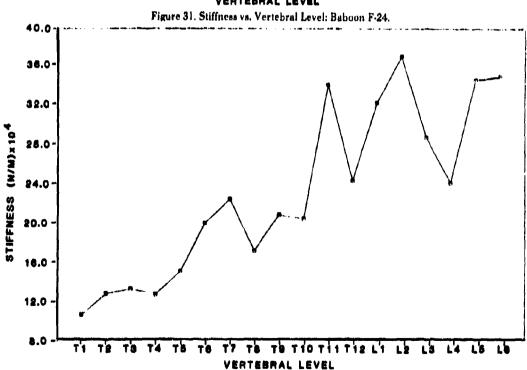


Figure 32, Stiffness vs. Vertebral Level: Baboon F-32.

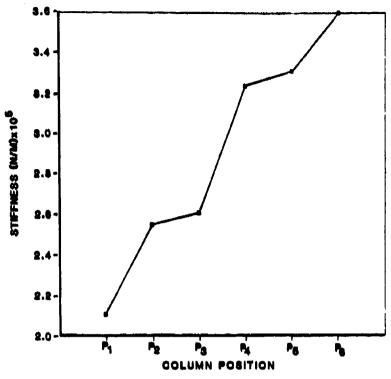


Figure 33. Stiffness vs. Column Position: Baboon F-24.

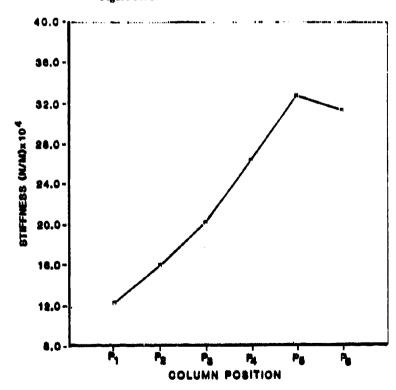


Figure 34. Stiffness vs. Column Position: Baboon F-32.

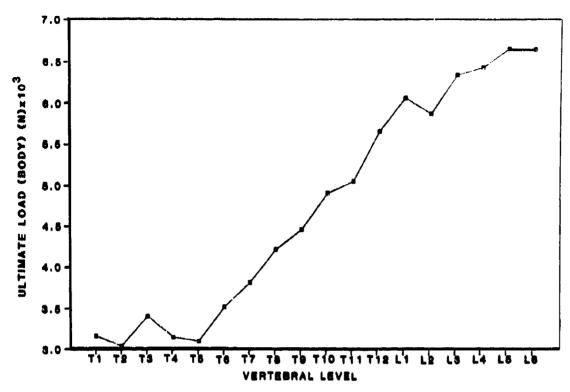


Figure 35. Ultimate Load vs. Vertebral Level: Baboon F-24.

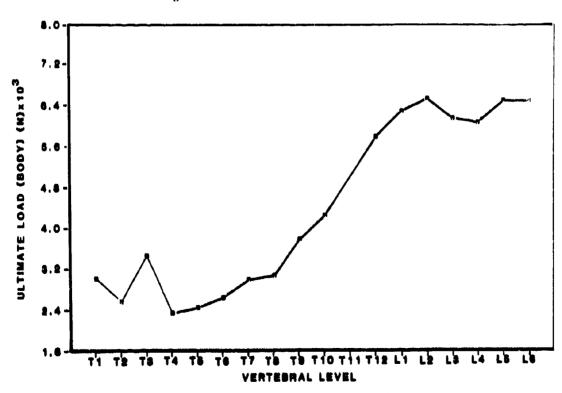


Figure 36. Ultimate Load vs. Vertebral Level: Baboon F-32.

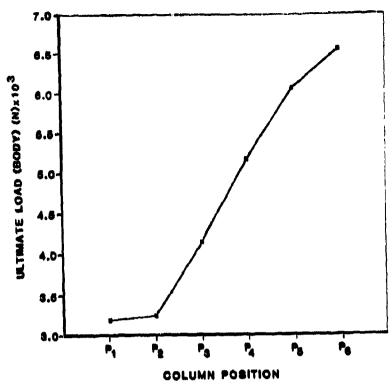


Figure 37. Ultimate Load vs. Column Position: Baboon F-24.

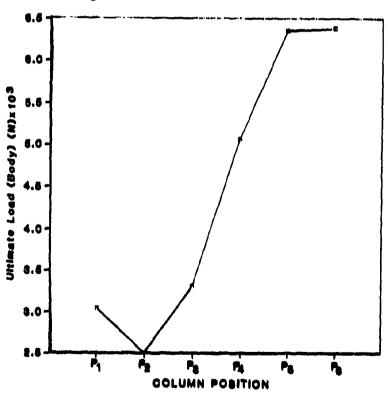


Figure 38. Ultimate Load vs. Column Position: Baboon F-32.

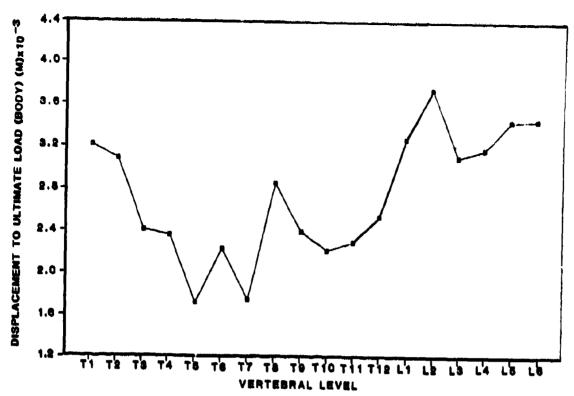


Figure 39. Displacement to Ultimate Load vs. Vertebral Level: Baboon F-24.

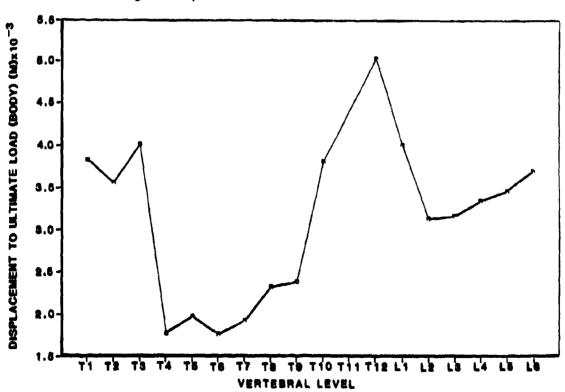
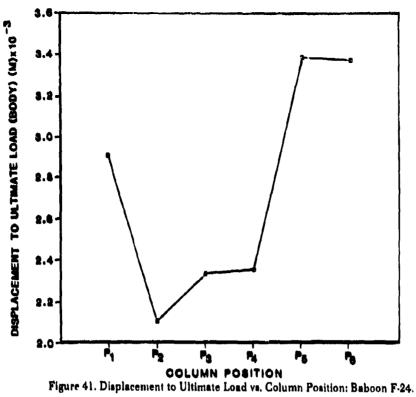


Figure 40. Displacement to Ultimate Load vs. Vertebral Level: Baboon F-32.



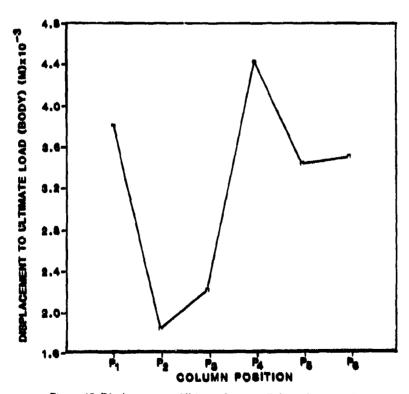


Figure 42. Displacement to Ultimate Load vs. Column Position: Baboon F-32.

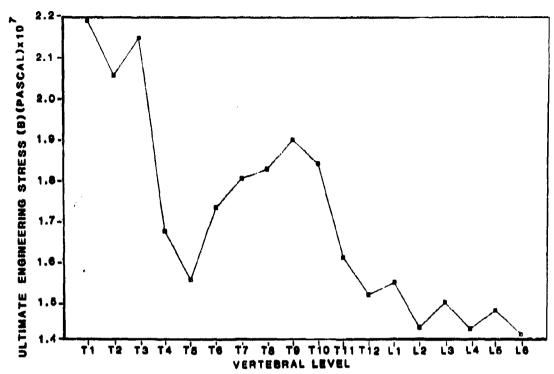


Figure 43. Ultimate Engineering Stress vs. Vertebral Level: Baboon F-24.

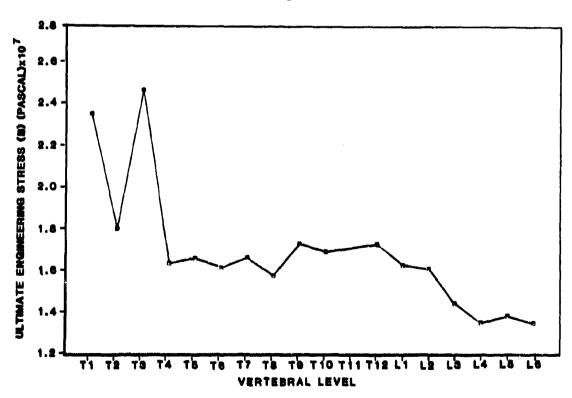


Figure 44. Ultimate Engineering Stress vs. Vertebral Level: Baboon F-32.

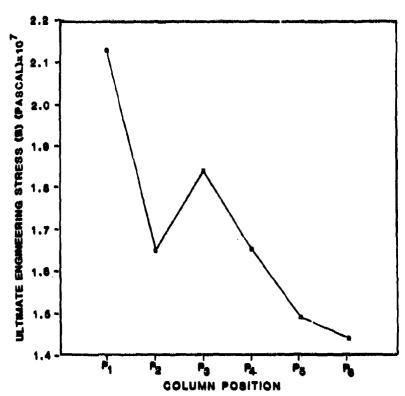


Figure 45. Ultimate Engineering Stress vs. Column Position: Baboon F-24.

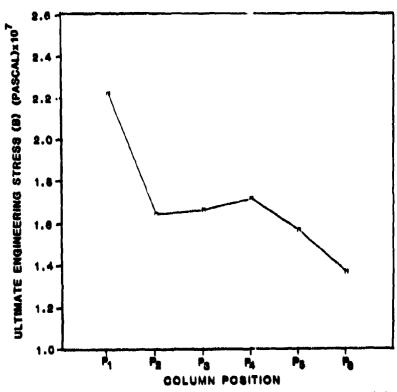


Figure 46. Ultimate Engineering Stress vs. Column Position: Baboon F-32.

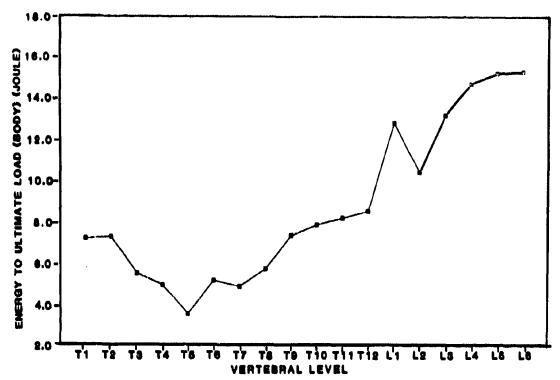


Figure 47. Energy to Ultimate Load vs. Vertebral Level: Baboon F-24.

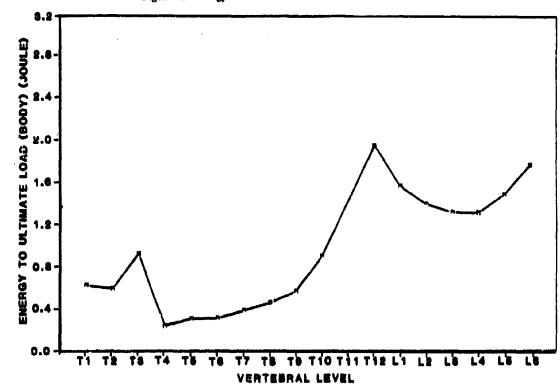


Figure 48. Energy to Ultimate Load vs. Vertebral Level: Baboon F-32.

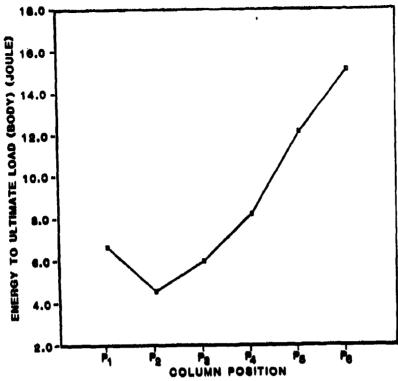


Figure 49. Energy to Ultimate Load vs. Column Position: Baboon F-24. ENERGY TO ULTIMATE LOAD (BODY) (JOULE) 16.0 14.0 12.0 10.0 8.0 8.0 4.0 COLUMN POSITION
Figure 50. Energy to Ultimate Load vs. Column Position: Baboon F-32.

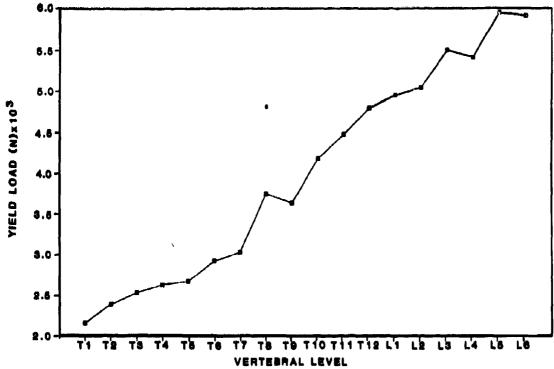


Figure 51. Yield Load vs. Vertebral Level: Baboon F-24.

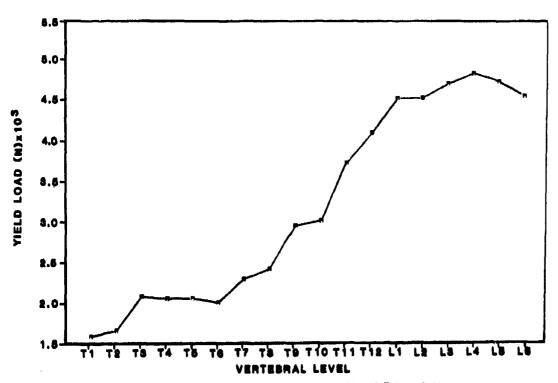


Figure 52. Yield Load vs. Vertebral Level: Baboon F-32.

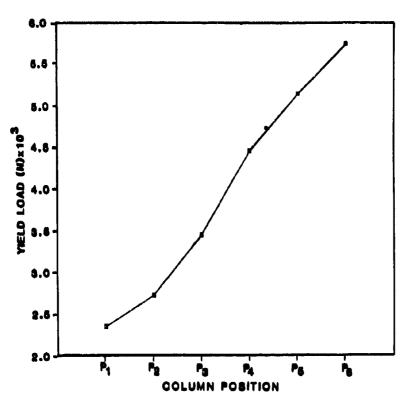


Figure 53. Yield Load vs. Column Position: Baboon F-24.

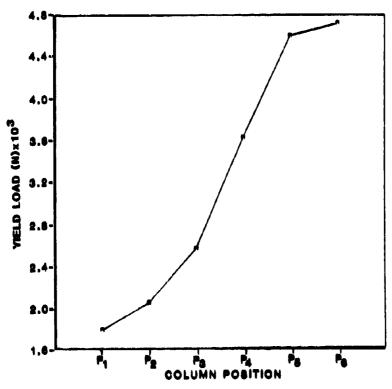


Figure 54. Yield Load vs. Column Position: Baboon F-32.

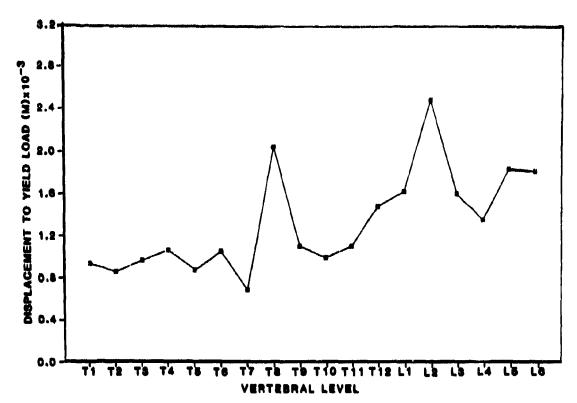


Figure 55. Displacement to Yield Load vs. Vertebral Level: Baboon F-24.

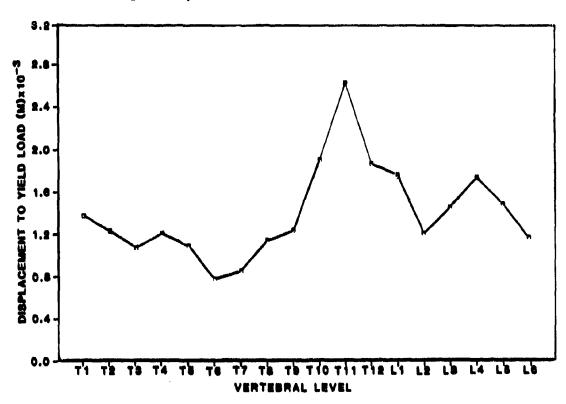


Figure 56. Displacement to Yield Load vs. Vertebral Level: Baboon F-32.

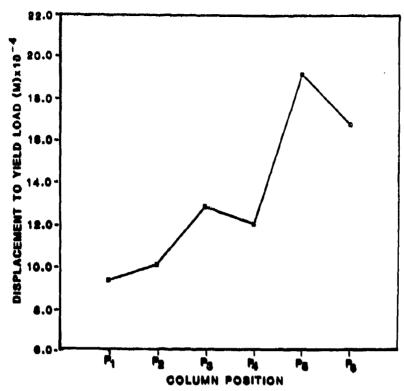


Figure 57. Displacement to Yield Load vs. Column Position: Baboon F-24.

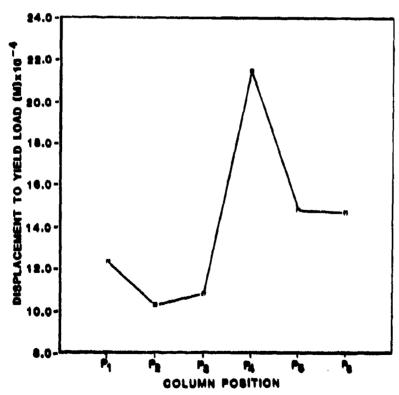
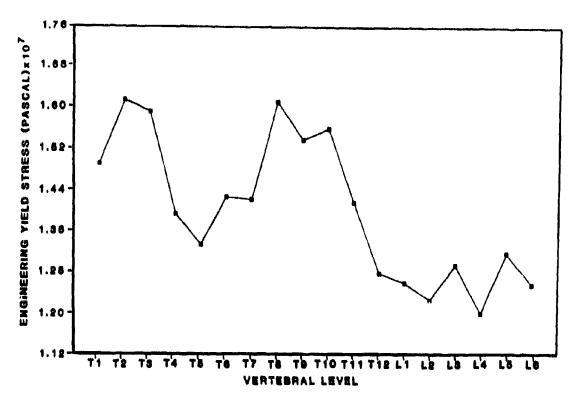


Figure 58. Displacement to Yield Load vs. Column Position: Baboon F-32.



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Figure 59. Engineering Yield Stress vs. Vertebral Level: Baboon F-24.

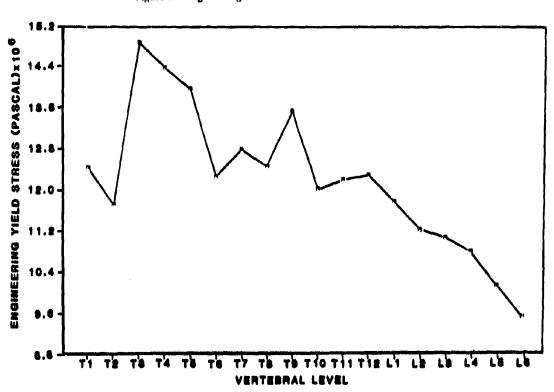


Figure 60. Engineering Yield Stress vs. Vertebral Level: Baboon F-32.

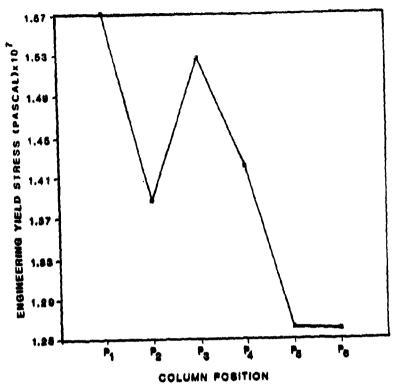


Figure 61. Engineering Yield Stress vs. Column Position: Baboon F-24.

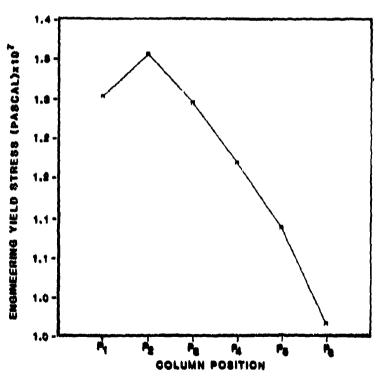


Figure 62. Engineering Yield Stress vs. Column Position: Baboon F-32.

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